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Correction of glucose intolerance and the impaired insulin release of chronic renal failure by verapamil

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Correction of glucose intolerance and the impaired insulin release of chronic renal failure by verapamil. Insulin release from pancreatic islets is impaired in chronic renal failure (CRF), and this is due to the state of secondary hyperparathyroidism of CRF. This defect in association with resistance to the peripheral action of insulin-caused glucose intolerance in CRF. It has been suggested that the impaired insulin release induced by excess parathyroid hormone (PTH) is related to the ability of the hormone to augment calcium entry into the pancreatic islets, resulting in accumulation of calcium in the pancreas. Therefore, a calcium channel blocker may antagonize this effect of PTH, and hence normalize glucose tolerance in CRF. The present study examined this postulate by studying intravenous glucose tolerance and insulin release from pancreatic islets in normal and CRF rats and in CRF animals treated with the calcium channel blocker, verapamil. Rats with 42 days of CRF displayed impaired glucose tolerance, significant reduction ($P < 0.01$) in insulin release by islets, and doubling of calcium content of the pancreas ($P < 0.01$) as compared to normal rats. Simultaneous treatment of CRF rats with verapamil for 42 days resulted in normal glucose tolerance, higher blood insulin levels during glucose infusion, normal calcium content of the pancreas, and normal insulin secretion by the islets. Treatment of normal rats with verapamil for 42 days did not affect any of the parameters studied. The results show that the calcium channel blocker, verapamil, by preventing calcium accumulation in the pancreas, reversed the abnormalities in insulin release that occur in CRF. This effect allowed a greater rise in blood levels of insulin during glucose infusion in CRF rats. These higher levels of insulin overcame the peripheral resistance to its action, and hence normalized glucose tolerance in CRF.

Carbohydrate intolerance is common in patients [1] and animals [2, 3] with chronic renal failure. This is due to peripheral resistance to the action of insulin [4] and to inappropriate insulin secretion in response to the insulin resistant state [1, 5, 6]. We [2] and others [6, 7], utilizing euglycemic and hyperglycemic clamp techniques, found that the state of secondary hyperparathyroidism of CRF [2, 8, 9] impairs the ability of the pancreas to secrete insulin but does not affect the resistance to the peripheral action of this hormone. Hence, excess blood levels of parathyroid hormone (PTH) play an important role in the genesis of carbohydrate intolerance of CRF through the action of the hormone on insulin release. Indeed, prevention or correction of hyperparathyroidism in CRF by parathyroidectomy [2, 6] or by medical suppression of the parathyroid gland activity [7] was associated with normalization of the glucose intolerance.

Additional studies from our laboratory [3] demonstrated that insulin secretion from perfused pancreatic islets obtained from rats with CRF is indeed impaired. In contrast, islets from parathyroidectomized CRF rats secreted insulin similar to normal islets. In addition, impaired insulin secretion was displayed by islets from rats with normal renal function treated with intact PTH. These observations clearly documented a direct effect of excess PTH on insulin secretion by the pancreatic islets, and further showed that this action of PTH may be related to the PTH-induced calcium accumulation in the pancreas.

If this is the case, one should be able to normalize pancreatic insulin secretion and reverse glucose intolerance of CRF by preventing calcium accumulation in the pancreas even if the state of secondary hyperparathyroidism is not corrected. Such a goal may be achieved by the use of a calcium channel blocker. The present study was undertaken to examine the effects of chronic treatment with the calcium channel blocker, verapamil, on glucose tolerance of rats with CRF and on insulin release by the pancreatic islets of these rats.

Method

Male Sprague-Dawley rats weighing 275 to 350 g were studied. They were fed normal rat chow diet (ICN nutritional Biochemical, Cleveland, Ohio, USA) throughout the study and allowed to drink ad libitum. Experiments were performed in four groups of animals: a) normal rats (normal), b) normal rats receiving verapamil (normal-VER), c) rats with chronic renal failure (CRF), and d) CRF rats receiving verapamil (CRF-VER). CRF was produced by two-stage 5/6 nephrectomy. The animals underwent right partial nephrectomy through a flank incision; a week later, a left nephrectomy was performed. Verapamil (0.1 μg/g body wt) dissolved in saline was given to one group of normal animals and of CRF rats twice daily through subcutaneous injections. The other normal and CRF rats received subcutaneous injections twice daily of vehicle (saline) only. Previous studies in our laboratory showed that glucose intolerance and abnormal insulin release occurred after

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six weeks of CRF [3]. Therefore the experiments in the present study were performed after 42 days of CRF or verapamil treatment.

In one part of the study, a one-hour intravenous glucose tolerance test (IVGTT) was done in normal, normal-VER, CRF and CRF-VER rats. The animals were not fasted before the test. The jugular vein and carotid artery were cannulated with PE 10 tubing under general anesthesia with ketamine-HCl 75 mg/kg body wt (Bristol Laboratories, Syracuse, New York, USA). The animals were allowed to recover from the surgical procedure and were studied 12 hours later in the awake state. The rats received 0.5 g of D-glucose/kg body wt in a bolus intravenous injection. A total of eight blood samples of 60 μl each were collected serially before and at 5, 15, 30, 45 and 60 minutes after glucose injection from the arterial line for the measurement of glucose and insulin.

In another part of the study, the animals were sacrificed by decapitation and the pancreas was dissected free of adipose tissue. The islets were isolated by the collagenase digestion method of Lacy and Kostianovsky [10] and picked free of exocrine tissue under a dissecting microscope. Both static and dynamic studies were done according to methods previously described [11, 12].

Briefly, in the static studies the islets were preincubated for a period of 30 minutes at 37°C in a modified Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM HEPES and 0.5 mg/dl bovine serum albumin (incubation media) and 2.8 mM D-glucose. The islets were then matched for size by visual inspection and groups of 10 islets were incubated in tubes containing 1.0 ml of the incubation media and were studied with the following secretogogues: a) 2.8 mM D-glucose, b) 16.7 mM D-glucose, c) 100 μM isobutyl-1-Methylxanthine (IBMX) and 16.7 mM D-glucose, and d) 10 μM forskolin and 16.7 mM D-glucose. After 30 minutes of incubation in a shaker bath at 37°C, the supernatants were aspirated for determination of insulin.

The dynamic studies were conducted in a four channel perifusion apparatus utilizing previously described methods [11, 12]. Twenty-five size-matched islets were placed in each of the four conical chambers of 0.07 ml capacity and were perifused at a rate of 0.8 ml/min with the incubation media containing 2.8 mM D-glucose at a temperature of 37°C and a gas mixture of 95% O_2 and 5% CO_2 being continuously bubbled into the perifusate. After leaving the chambers, the perfusate was filtered through 8.0 μm pore size filter (Sartorius Burlingame, California, USA) and was collected. Each study was performed in duplicates. After 39 minutes of pre-incubation, the collection of the effluent was started and continued at a one minute interval for 41 minutes. The first six collections (6 min) represented the basal level of insulin release during perifusion with 2.8 mM D-glucose. Thereafter, the D-glucose concentration in the perifusate was increased to 16.7 mM and an additional 35 samples were collected. Insulin concentration was then determined in the various samples of the effluent.

In the dynamic studies of insulin release, the changes from baseline with time were examined by calculating the area under the curve for each study. Insulin release started to increase four minutes after the change of the concentration of D-glucose in the perifusate to 16.7 mM. Therefore, the average values of insulin release during the six minutes prior to the change in glucose concentration and the four minutes immediately thereafter were used as a baseline level. The calculation of the areas under the curve allowed us to estimate insulin release during the initial phase (5 min between min 4 and 9) and the total insulin release (31 min between min 4 and 35).

Calcium content of the pancreas was also measured. About 0.5 to 1.0 g of pancreas was placed in tarred porcelain crucibles and weighed to 0.01 mg. All samples were dried at 105°C for 48 hours and then reweighed to determine water content. Samples were then ashed for 12 hours in an oven with 700°C. The samples were then extracted in 0.75 N HNO_3 for 24 hours and calcium concentration was determined. The measurement of calcium was made with Perkin Elmer atomic absorption spectrophotometer, Model 503 (Perkin Elmer Corp., Norwalk, Connecticut, USA), those of creatinine and phosphorus with Technicon autoanalyzer (Technicon Instrument Inc., Tarrytown, New York, USA), and those of glucose by glucose oxidase method utilizing Beckman glucose analyzer II (Beckman Instrument, Inc., Fullerton, California, USA). Insulin was determined by charcoal-coated radioimmunoassay using rat insulin as standard [13].

Statistical analysis was done with the Clinfo computer system. The data were presented as mean ± SE. Changes from baseline in parameters with mutiple measurements with time (plasma, glucose, and insulin during IVGTT and dynamic insulin release) were evaluated by calculating area under the curve for each experiment utilizing the trapezoidal rule. The areas under the curve as well as the data from studies of static insulin release were analyzed by one-way analysis of variance and compared with each other using the Duncan multiple range test. Non-parametric analysis was also done using Wilcox non-paired rank sum tests adjusted for multiple comparison.

Results

IVGTT studies

Table 1 provides the data on blood chemistry for the animals which were studied with IVGTT and Figures 1 and 2 show the changes in plasma glucose and insulin during the 60 minutes of the test, respectively. The nephrectomy procedure resulted in a significant ($P < 0.01$) rise in the plasma levels of creatinine with the values being three times higher than in normal or normal-VER rats. There were no significant differences in the plasma levels of calcium and phosphorus among the various groups of rats. The plasma levels of potassium in CRF rats were significantly ($P < 0.01$) higher than those of the other three groups. The body weight of CRF-VER rats was lower than normal but it was not significantly different from that of CRF rats.

Within five minutes after the injection of glucose load, the plasma concentration of glucose reached their peak and decreased thereafter. The rats with CRF displayed glucose intolerance (Fig. 1) with the area under the curve for plasma glucose significantly ($P < 0.01$) higher than that of the other three groups of animals (Table 1). The treatment of CRF rats with verapamil was associated with a normal glucose tolerance test and the area under the curve for plasma glucose in the CRF-VER was not different from that in normal or normal-VER rats. Verapamil did not affect glucose tolerance in normal animals.

There were significant increments in plasma insulin levels in all groups of animals (Fig. 2). There was no significant differ-

Table 1. Body weight, blood chemistry and area under the curve for plasma glucose and insulin in the various groups of rats studied with intravenous glucose tolerance test

	Body weight	Creatinine	Plasma calcium	Phosphorus	Potassium	Area under the IVGTT curve	
						plasma glucose	plasma insulin
	g	*mg/dl*				*mg/dl · 60 min*	*pg/ml · 60 min*
Normal $N = 4$	398 ± 8	0.55 ± 0.01	9.5 ± 0.48	6.9 ± 0.30	4.20 ± 0.10	6528 ± 468	75 ± 5
Normal-VER $N = 5$	369 ± 13	0.52 ± 0.02	9.9 ± 0.33	6.0 ± 0.11	4.25 ± 0.12	6143 ± 144	79 ± 1
CFR $N = 4$	350 ± 13	1.58 ± 0.09[a]	10.4 ± 0.33	6.4 ± 0.50	5.30 ± 0.09[b]	9065 ± 199[b]	118 ± 16[b]
CRF-VER $N = 4$	332 ± 7[a]	1.66 ± 0.04[a]	10.3 ± 0.32	6.6 ± 0.28	4.15 ± 0.15	6990 ± 350	185 ± 18[b]

Data are presented as mean ± SE.
[a] $P < 0.01$ vs. normal and normal-VER
[b] $P < 0.01$ vs. all other groups

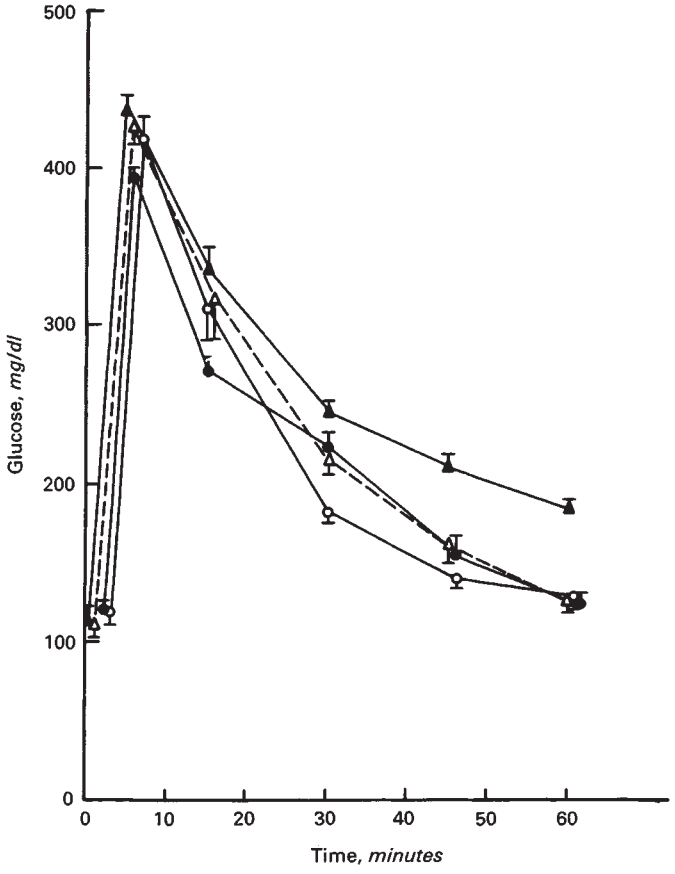

Fig. 1. *The changes in plasma glucose concentration during intravenous glucose tolerance tests.* Each data point represents mean value and brackets denote 1 SE. Symbols are: (○) normal; (●) normal + verapamil; (▲) CRF; (△) CRF + verapamil.

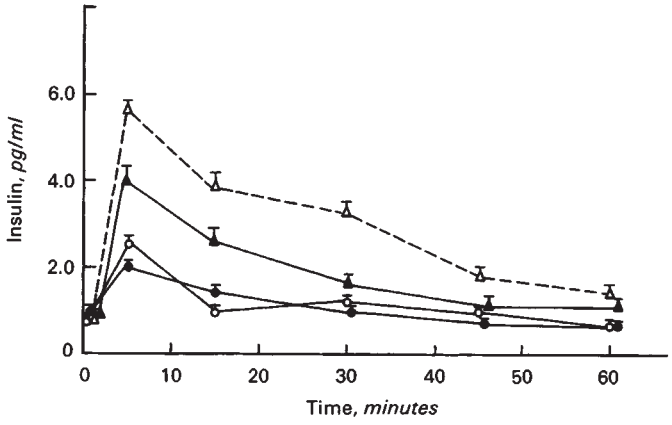

Fig. 2. *The changes in plasma levels of insulin during intravenous glucose tolerance test.* Each data point represents mean value and brackets denote 1 SE. Symbols are (○) normal; (●) normal + verapamil; (▲) CRF; (△) CRF + verapamil.

ence in the changes in the plasma insulin levels between normal and normal-VER rats. In the CRF animals, plasma insulin concentrations increased from 0.9 ± 0.06 pg/ml to a peak of 4.0 ± 0.67 pg/ml and remained elevated to a lesser degree throughout the study. In the CRF-VER rats, the maximum increment in plasma insulin concentration (from 0.8 ± 0.06 to 5.80 ± 0.41 pg/ml) was significantly ($P < 0.01$) higher than that observed in CRF rats. The levels then gradually decreased but were always higher than those in CRF rats. The area under the curve for plasma insulin in the studies in CRF-VER was also significantly ($P < 0.01$) higher than in CRF rats (Table 1).

Insulin release by pancreatic islets

The biochemical data, insulin release during static studies, calcium content of the pancreas and insulin secretion during dynamic studies in normal, normal-VER, CRF and CRF-VER rats are shown in Table 2 and Figures 3 and 4. The CRF and CRF-VER rats had significantly ($P < 0.01$) higher plasma levels of creatinine than normal and normal-VER rats. There were no significant differences between the plasma concentrations of calcium and phosphorus among the various groups of animals.

Insulin release induced by 16.7 mM D-glucose during static studies in CRF rats (51 ± 2 pg/islet/min) was significantly ($P < 0.01$) lower than that in normal (148 ± 13 pg/islet/min), normal-VER (151 ± 14 pg/islet/min) and CRF-VER (115 ± 4 pg/islet/min) (Fig. 3). Both IBMX and forskolin produced significant ($P < 0.01$) rise in insulin secretion, and the increments over that produced by 16.7 mM glucose alone were not significantly different (Fig. 3) in the four groups of rats.

Insulin release during the dynamic studies is shown in Figure 4. It is evident that insulin release in CRF rats was lower than normal, normal-VER, and CRF-VER rats. Indeed the areas under the curve for both the early and total insulin release in

Table 2. Body weight, blood chemistry, insulin release and pancreatic calcium content in various groups of rats studied with the isolated perfused islet technique

	Body weight *g*	Plasma *mg/dl*			Insulin release *pg/islet*		Pancreatic calcium *g/kg dry wt*
		Cr	Ca	P	Phase 1 (5 min)	Total (31 min)	
Normal $N = 6$	389 ± 9	0.55 ± 0.05	9.5 ± 0.48	6.3 ± 0.80	323 ± 39	3816 ± 221	4.7 ± 0.15
Normal-VER $N = 6$	396 ± 11	0.52 ± 0.02	9.2 ± 0.32	6.5 ± 0.70	339 ± 19	3748 ± 206	4.5 ± 0.28
CRF $N = 5$	353 ± 11[a]	1.89 ± 0.19[a]	9.5 ± 0.32	5.9 ± 0.52	42 ± 13[b]	1073 ± 208[b]	10.8 ± 0.90[b]
CRF-VER $N = 6$	329 ± 12[a]	1.87 ± 0.16[a]	9.7 ± 0.33	6.2 ± 0.74	170 ± 25[a]	3045 ± 288	5.1 ± 0.44

Data are presented as mean ± SE. Abbreviations are: Cr, creatinine; Ca, calcium; P, phosphorus.
[a] $P < 0.01$ vs. normal and normal-VER
[b] $P < 0.01$ vs. all other groups

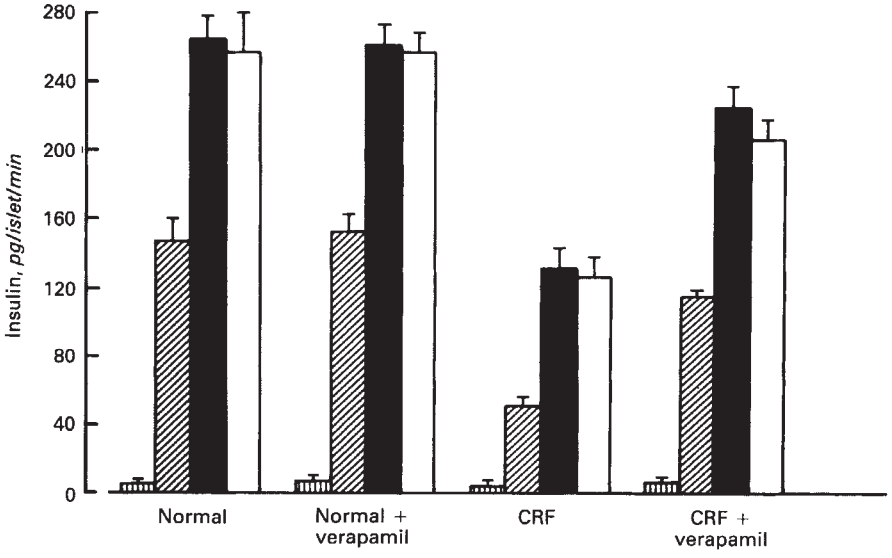

Fig. 3. *Insulin release from pancreatic islets during studies with static incubation.* Each column represents mean value and brackets denote 1 SE. Symbols are: (▥) 2.8 mM glucose; (▨) 16.7 mM glucose; (■) 16.7 mM glucose + 100 μM IBMX; (□) 16.7 mM glucose + 10 μM forskolin.

CRF rats were significantly ($P < 0.01$) lower than those in the other three groups of animals (Table 2). Although treatment of CRF rats with verapamil was associated with a significantly higher first-phase insulin release, the values were still significantly ($P < 0.01$) lower than those in normal or normal-VER animals; however, total insulin release in CRF-VER rats was not different from normal or normal-VER animals. The calcium content of the pancreas in CRF rats was significantly ($P < 0.01$) higher than that of normal, normal-VER and CRF-VER animals. There were no significant differences in the calcium content of the pancreas among the latter three groups of animals. Thus, treatment of CRF animals with verapamil prevented the accumulation of calcium in the pancreas.

Discussion

The results of the present study demonstrate that treatment of CRF rats for six weeks with verapamil corrected the glucose intolerance. This conclusion implies that the area under the curve for plasma glucose during IVGTT in CRF rats treated with verapamil is not different from that of normal rats treated with this drug. Indeed, analysis of the data confirm the absence

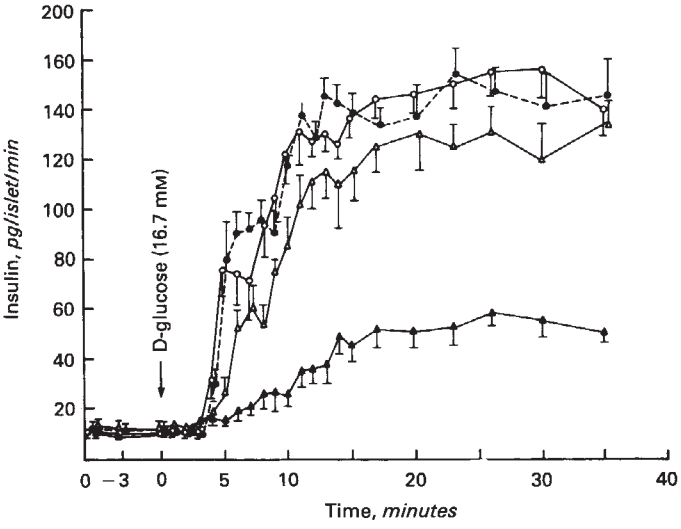

Fig. 4. *Dynamic insulin release from perfused pancreatic islets.* Each data point depicts mean value and brackets represents 1 SE. Symbols are: (○) normal; (●) normal + verapamil; (▲) CRF; (△) CRF + verapamil.

of a statistically significant difference between the two groups. However, the mean value in CRF rats treated with verapamil was somewhat higher than that of the normal animals receiving verapamil. It is possible, therefore, that the treatment with verapamil did not completely correct the glucose intolerance of CRF rats but rather produced marked and significant improvement in this abnormality.

This effect of verapamil is most likely due to the higher blood levels of insulin after the intravenous glucose injection. These observations are similar to those reported by us in parathyroidectomized CRF dogs [2] and in CRF humans in whom the activity of the parathyroid glands was suppressed medically or in those subjected to PTX [6, 7]. Thus, it appears that reduction of PTH levels in animals or humans with CRF by medical or surgical means [6, 7], or that the chronic treatment of CRF rats with verapamil which does not affect serum levels of PTH [14] permits the pancreas to produce more insulin; such a response is sufficient to overcome the resistance to the peripheral action of insulin and results in normal glucose tolerance.

Our studies were not designed to examine the effect of verapamil on the peripheral resistance to the action of insulin in CRF. However, our data indicate that CRF-VER rats had a two- to fivefold increase in serum insulin levels during IVGTT and normal glucose tolerance as compared to 50% increase in plasma insulin levels in CRF rats who had abnormal glucose tolerance. These data clearly suggest that verapamil did not improve the peripheral resistance to insulin and may even have worsened it. However, if chronic verapamil administration is associated with the development or worsening of peripheral resistance to insulin action, one would expect that glucose intolerance develops in the normal-VER animals. This did not occur and these animals had normal glucose tolerance. The issue of the effect of verapamil treatment on peripheral resistance to insulin could be evaluated by euglycemic insulin clamp technique, which was not done in our studies.

Most available data indicate that the peripheral resistance to insulin is not due to alterations in the number or affinity of insulin receptors but rather due to a post-receptor defect [15]. Since our data is consistent with the notion that the effect of verapamil on glucose intolerance in CRF is not mediated through improvement in the peripheral resistance to insulin, one may assume that the therapy does not improve the post-receptor defect.

The results of the static studies with forskolin and IBMX indicate that the reduction in insulin secretion from islets of CRF rats and the correction of this defect by treatment with verapamil is not related to an effect on the adenylate cyclase-cyclic AMP system. An increase in cyclic AMP produced either by forskolin, which stimulates adenylate cyclase activity [16], or by IBMX, which inhibits phosphodiesterase activity [17], caused a similar augmentation in insulin secretion by islets from normal, normal-VER, CRF and CRF-VER rats.

The plasma levels of insulin during IGVTT in CRF rats were higher than that in the normal animals, while insulin release from islets was lower in the former than in the latter group. This observation seems contradictory. However, it must be emphasized that plasma insulin levels represent the balance between the rate of secretion and that of degradation. Thus, the decreased degradation of insulin in CRF [18] is responsible for the higher insulin levels despite low insulin secretion. In CRF-VER rats, the higher insulin secretion from the islets combined with decreased degradation secondary to CRF are responsible for the significantly higher plasma insulin levels observed in these rats compared to the untreated CRF animals. It is, theoretically, possible that verapamil may alter insulin degradation but neither our study nor others refute or confirm such a possibility.

It has been suggested that excess PTH through its ability to enhance calcium entry into cells [19–25], causes accumulation of the calcium in the pancreatic islets [3], and such an effect could impair the function of these islets, resulting in reduced insulin release in response to glucose and hence glucose intolerance. We have previously reported [3], that the calcium content of the pancreas of CRF rats with intact parathyroid glands was significantly higher than that of the normal pancreas. The calcium content in these CRF rats was normalized by PTX. These changes in pancreatic calcium correlated with the insulin release which was impaired from islets of CRF rats but normal from islets of PTX-CRF animals.

The data of the present study provide further support for this notion. Again, the calcium content of the pancreas from CRF rats with intact parathyroid glands was significantly higher than normal and insulin release from the islets of these animals was markedly impaired. Chronic treatment of the CRF rats with verapamil, which does not affect PTH levels [14], prevented the PTH-induced calcium accumulation in the pancreas by blocking calcium entry and normalized the glucose-induced insulin release from the pancreatic islets.

Available data indicate that an acute rise in cytosolic calcium of islets induces insulin secretion [17, 26–28], and this appears to vitiate the notion that an increase in pancreatic calcium inhibited insulin secretion in our studies. However, it is possible that acute exposure of the pancreatic islets to PTH stimulates glucose-induced insulin release, but chronic exposure to the hormone and a consequent overloading of the islets with calcium exerts an inhibitory effect. A corollary to a different effect of acute or chronic excess of PTH is found in observations in other systems. The hormone acutely stimulates both the chronotropic [25] and the inotropic [29] properties of heart cells and enhances random migration of polymorphonuclear leucocytes [30], but chronic exposure to excess PTH decreases or stops the beating of heart cells [25], impairs the metabolism and function of the heart [31] and reduces random migration of the leucocytes [30].

We suggest that the effect of changes in cytosolic calcium of the islets on insulin release depends on the magnitude of these changes and their duration. Such a proposition would imply that a large and sustained increment in islet calcium could inhibit insulin release. If the increase in total pancreatic calcium found in our rats with CRF reflects a higher calcium content in the cells of both the exocrine and endocrine tissue of the pancreas, one may assume that calcium overloading of the islets is present. Under such circumstances, the capacity of the cellular organelles to sequester calcium and buffer the calcium load may approach saturation and a new steady state with higher cytosolic calcium may develop; in such a setting insulin release may be inhibited. Indeed, certain data are consistent with the notion that higher islets cytosolic calcium may reduce insulin release. Frankel, Atwater and Grodsky [32] demonstrated that high cytosolic calcium in pancreatic islets may activate potassium permeability, cause repolarization of the cell membrane and

blunt insulin release. Also it is known that glucose-induced insulin release will plateau as cytosolic calcium reaches a certain level [17]; thus, if the islets of CRF rats have higher cytosolic calcium than those of normal rats, the critical levels of cytosolic calcium in islets at which glucose-induced insulin secretion would plateau could be reached earlier and insulin release would be limited. Verapamil, by correcting calcium accumulation in the pancreas and presumably in the islets of CRF rats, would reverse the abnormalities in insulin release. Our proposition requires confirmation with direct measurements of cytosolic calcium.

The observations of the present study may provide a therapeutic approach for the control of glucose intolerance of CRF. However, it must be mentioned that in our study verapamil was given simultaneously with the induction of CRF. It is possible that verapamil may not correct glucose intolerance if it is given to animals or humans with established CRF. In such a situation, it may not be possible to reverse the changes in pancreatic calcium. However, Mak et al. [6, 7] were able to normalize glucose intolerance in CRF patients after six months of medical suppression of hyperparathyroidism or PTX, suggesting that reversal of the pancreatic abnormality is possible. We know of one study in which dialysis patients were treated for nine weeks with another calcium channel blocker, nifedipine [33]. Their blood levels of insulin during the oral glucose tolerance test were higher than in the patients receiving placebo, but the difference did not reach statistical significance. It is possible that a longer period of treatment with the drug is needed to achieve complete correction of the glucose intolerance by normalization of the pancreatic response to glucose.

It is well established that verapamil added in vitro to pancreatic islets inhibits insulin release by these structures [27, 34]. However, the effects of the in vivo administration of the drug may differ from those observed in the in vitro experiments. Indeed, several studies have shown that acute intravenous infusion of verapamil or oral administration of the drug for several days did not affect blood levels of insulin during glucose tolerance tests [35–37]. Others reported a decrease in blood insulin levels during intravenous infusion of verapamil in humans [38], dogs [39], or rats [40]. It is very difficult to compare these data describing the effects of acute in vivo administration of verapamil with our studies which evaluated the chronic in vivo effect of the drug in animals of CRF. Furthermore, our results showed that the chronic administration of verapamil to normal rats does not affect glucose tolerance, blood levels of insulin or insulin release from the islets of these animals.

Insulin release by the pancreas may be affected by changes in the concentrations of plasma calcium [41, 42], phosphorus [43, 44] and potassium [45]. An effect of verapamil on these parameters may influence insulin release independently of changes in pancreatic calcium. The differences between the plasma insulin levels during IVGTT and insulin release from islets between the normal, CRF, CRF-VER, and normal-VER could not be due to differences in the plasma concentrations of calcium and phosphorus since these two latter parameters were not different among the various groups. The lower plasma potassium concentration in the CRF-VER is most likely due to an effect of verapamil on extrarenal homeostasis of potassium [46]. This difference in plasma potassium between CRF-VER and CRF rats could not explain the effect of verapamil on glucose intolerance and insulin release in CRF-VER rats. The higher potassium level in the CRF rats should have been associated with higher insulin and not lower release since hyperkalemia stimulates insulin secretion.

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